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(54) Title: HEPATITIS C NS3 PROTEASE INHIBITORS

(57) Abstract

Disclosed is a specific pharmacophoric profile which represents the structure for inhibitors of Hepatitis C NS3 protease. The results from mapping studies of the enzyme with depsipeptide substrates allow the generation of a particular pharmacophoric binding profile. Compounds possessing this motif were shown to be inhibitors of Hepatitis NS3 protease. The inhibitors have use in the treatment of hepatitis C.

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Hepatitis C NS3 Protease Inhibitors

The present invention relates to inhibitors of hepatitis C NS3 protease, compounds which fit the pharmacophoric profiles of these inhibitors and use of these inhibitors for the treatment of hepatitis C.

Field of the invention

An analysis of the epidemiologic evidence suggests that there may be three types of non-A non-B hepatitis (NANBH); the water borne epidemic type, the blood or needle associated type, and the sporadically occurring (community acquired) type. It is thought that the recently discovered hepatitis C virus (HCV) infects over 300 million people worldwide and is the major etiological agent of both parenterally transmitted and sporadic NANBH.[1,2] Upon first exposure to HCV approximately 2-% of infected individuals develop acute clinical hepatitis while others appear to resolve spontaneously. However, in most instances the virus establishes a chronic infection which persists for decades.[4] This situation usually results in recurrent and progressively worsening liver inflammation, often leading to more severe disease states such as cirrhosis and hepatocellular carcinoma.[3]

Current treatment with interferon-α appears only to cause remission in 25% of patients, although it is also associated with a decrease in liver cell dysfunction and worsening of the disease. Of those patients who do respond they subsequently relapse when the drug is withdrawn. We believe that there is a clear need for orally active compounds which lower viral load and prevent viral replication of HCV.

The HCV virion has a positive strand RNA genome that contains a single large open reading frame encoding a polyprotein of 3010-3033 amino acid residues. The nonstructural proteins involved in replication of the HCV genome are released by the action of two proteinases; NS2-3 and NS3. The action of NS3 protease yields the nonstructural proteins NS4A, NS4B, NS5A and NS5B. The N-terminal domain of NS3 contains a trypsin-like serine protease and the catalytic triad of His-57, Asp-81 and Ser-139 (numbering from the start of NS3) are strictly conserved among all HCV derived

sequences. It is thought that, because the NS3 protease is essential for viral replication, then inhibitors of this enzyme will be useful in the treatment of NANBH disease.

Description of the related art.

As far as we are aware only one, weakly active (2.5 $\mu g ml^{-1}$) small molecule inhibitor of Hep C NS3 has been previously reported in the literature.[5] Depsipeptides have previously been synthesised as substrates for hepatitis C NS3 in order to establish a high throughput assay, [6,7] but have not been previously used to generate a pharmacophoric profile that will be useful in designing inhibitors of Hepatitis C NS3 protease.

Summary of the invention

Mapping of HCV NS3 protease with a series of depsipeptide substrates of the type 6 (Scheme 1) provided sufficient data (Table 1) for the computer generation of a pharmacophoric profile of the pockets on the non-prime side of the catalytic centre. It is known in the art[10] that compounds of the type 12 (Scheme 2) which bind tightly to the non-prime sites of other serine proteases, and that do not possess a warhead which binds directly to the catalytic centre, can possess potent inhibitory activity. In view of this pharmacophoric profiles have been identified which describe inhibitors of hepatitis C NS3 protease. These pharmacophoric profiles are shown in figures 1,3 and 6. Therefore, compounds possessing a pharmacophoric profile of the type shown in Figures 1,3 and 6 will be inhibitors of hepatitis C NS3 protease and will be useful in the treatment of NANBH.

Moreover, judicious connection of all or some of the important residues required for binding to the non-prime P_1-P_5 sites of the NS3 protease[8,9] *via* a non peptide scaffold will allow the preparation of orally active drugs. Although some information of the preferred motifs can be gained from a knowledge of the natural cleavage data, somewhat surprisingly we have discovered that an aromatic containing residue, such as homophenylalanyl, 1,2,3,4,-D-tetrahydroisoquinoline, 3-pyridyl, tyrosine etc, in the P_3 position of the inhibitor is extremely beneficial for inhibitory activity.

In a first aspect the invention provides novel inhibitors of hepatitis C NS3 protease.

In a further aspect the invention provides compounds which fit a pharmacophoric profile of a hepatitis C NS3 protease inhibitor.

In a further aspect the invention provides use of inhibitors of the invention for the treatment of hepatitis C.

In a further aspect the invention provides use of the inhibitors of the invention in the manufacture of a medicament for the treatment of hepatitis C.

In a further aspect of the invention there is provided a method of treatment of hepatitis C which comprises administering a pharmaceutically active amount of an inhibitor of the invention.

In a further aspect the present invention provides a composition which comprises a pharmaceutically acceptable amount of an inhibitor of the invention together with a pharmaceutically acceptable carrier or diluent.

The invention will now be described with reference to the accompanying drawings in which:

Figure 1 shows a first pharmacophoric profile of a substrate of hepatitis C NS3 protease.

Figure 2 shows the distance and angle constraints of the pharmacophoric profile of figure 1.

Figure 3 shows a second pharmacophoric profile of a substrate of hepatitis C NS3 protease.

Figures 4 and 5 show the distance and angle constraints of the pharmacophoric profile of figure 3.

Figure 6 shows a third pharmacophoric profile of a substrate of hepatitis C NS3 protease.

Figure 7 shows the distance and angle constraints of the pharmacophoric profile of figure 6.

Figure 8 shows an example of a compound which fits the pharmacophoric profile of figure 1.

Figure 9 shows an example of a compound which fits the pharmacophoric profile of figure 3.

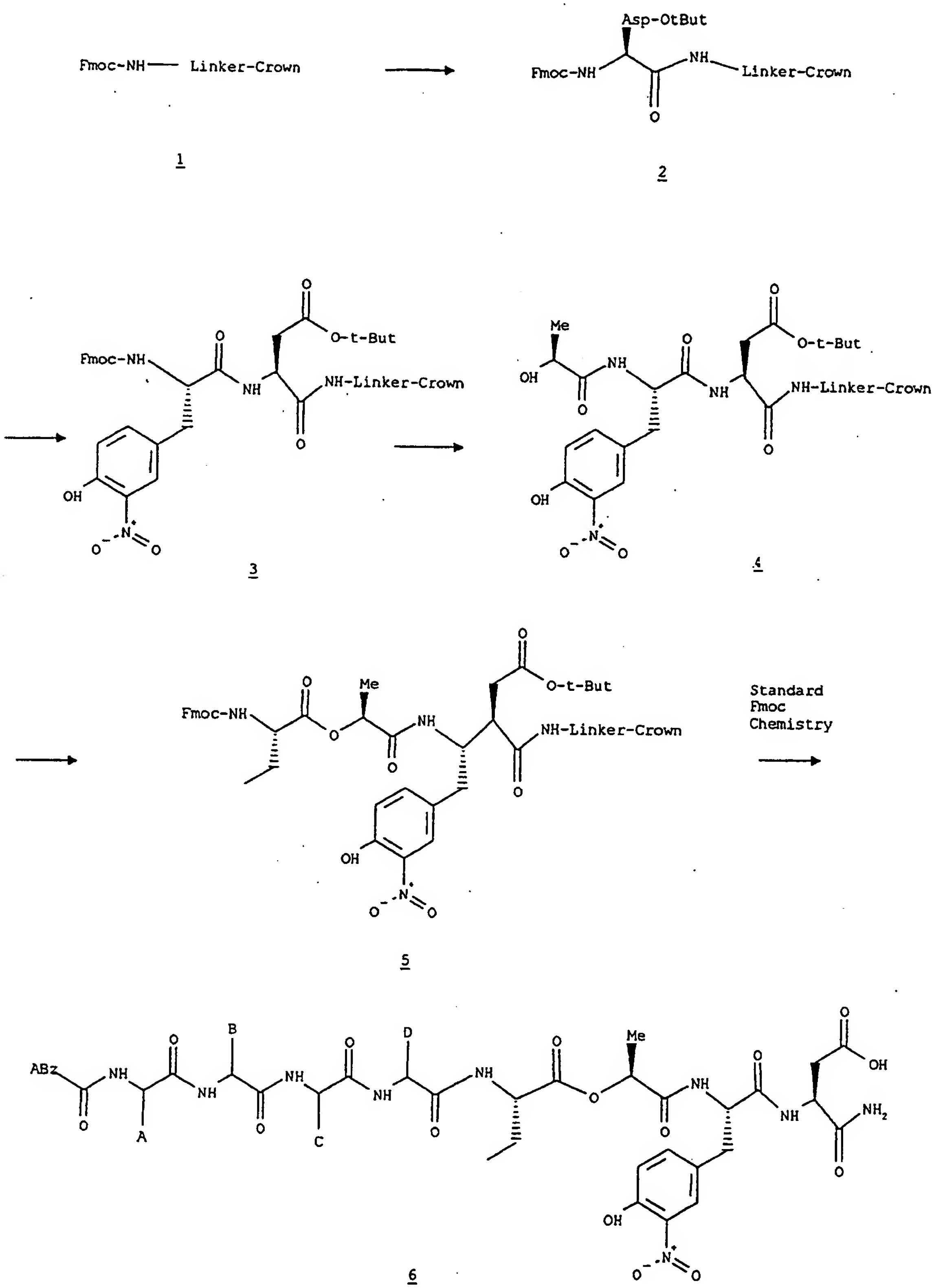
Figure 10 shows an example of a compound which fits the pharmacophoric profile of figure 6.

Experimental

NS3 folding and protease activity

Chemicals and biochemicals were of the highest available grade and unless otherwise stated purchased from Sigma Chemical Co., Poole, U.K.

Purified insoluble NS3 protease (15.6 mg/mL in 8 M urea) was obtained from the Hepatitis C J8 strain. Refolding of the NS3 protease was carried out by dilution of the protein to 100 nM into 50 mM Tris•HCl; pH 7.4 containing 10 mM CHAPS (Calbiochem, Nottingham, U.K.); 2 mM 2-mercaptoethanol; 10 mM magnesium sulphate and 50% glycerol. The first phase of the folding reaction was carried out at 4°C for 1 h and the solution was kept well stirred. After incubation, the sample was treated with NS4A cofactor (1 equivalent, with respect to the protease, of H-KKGSISIIGRLHLNDRVVVAPKK-OH) to form a 100 nM solution which was kept well stirred further incubated at room temperature (20°C) for a further 15 min. The protease solution was adjusted to 10 mM 2-mercaptoethanol and this solution was used for NS3 protease assays.


Fmoc-NH— Linker-Crown
1
Asp-OtBut
Fmoc-NH
NH
Linker-Crown
O
2
Fmoc-NH
O
O-t-But
NH
NH-Linker-Crown
OH
3
Me
OH
NH
4
Fmoc-NH
Me
O-t-But
NH-Linker-Crown
Standard
Fmoc
Chemistry
5
ABz
NH
A
B
C
D
OH
NH$_2$
6

Scheme 1

Synthesis of Depsipeptide Compounds

Depsipeptide substrates were synthesised according to the route shown in Scheme 1.

Preparation of Crown Assembly

The depsipeptide compounds were synthesised in parallel fashion using Fmoc-Rink-DA/MDA derivatised macrocrowns (ex Chiron Mimotopes, Australia) loaded at approximately 7 μM per crown. Prior to synthesis each crown was connected to its respective stems and slotted into the 8 x 12 stem holder. Coupling of the amino acids employed standard Fmoc amino acid chemistry as described in 'Solid Phase Peptide Synthesis', E. Atherton and R.C. Sheppard, IRL Press Ltd, Oxford, UK, 1989.

Removal of Nα-Fmoc Protection

A 250 ml solvent resistant bath is charged with 200 ml of a 20% piperidine/DMF solution. The multipin assembly is added and deprotection allowed to proceed for 30 minutes. The assembly is then removed and excess solvent removed by brief shaking. The assembly is then washed consecutively with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) and left to air dry for 15 minutes.

Quantitative UV Measurement of Fmoc Chromophore Release

A 1 cm path length UV cell is charged with 1.2 ml of a 20% piperidine/DMF solution and used to zero the absorbance of the UV spectrometer at a wavelength of 290nm. A UV standard is then prepared consisting of 5.0 mg Fmoc-Asp(OBut)-Pepsyn KA (0.08 mmol/g) in 3.2 ml of a 20% piperidine/DMF solution. This standard gives Abs_{290} = 0.55-0.65 (at room temperature). An aliquot of the multipin deprotection solution is then diluted as appropriate to give a theoretical Abs_{290} = 0.6, and this value compared with the actual experimentally measured absorbance showing the efficiency of previous coupling reaction.

Coupling of Standard Amino Acid Residues

Coupling reactions are performed by charging the appropriate wells of a polypropylene 96 well plate with the pattern of activated solutions required during a particular round of coupling. Macrocrown (approx 7 μ mole) standard couplings are performed in DMF (500 μl).

Coupling of an Amino-acid Residue To Appropriate Well

Whilst the multipin assembly is drying, the appropriate N_α-Fmoc amino acid pfp esters (10 equivalents calculated from the loading of each crown) and HOBt (10 equivalents) required for the particular round of coupling are accurately weighed into suitable containers. Alternatively, the appropriate N_α-Fmoc amino acids (10 equivalents calculated from the loading of each crown), desired coupling agent e.g. HBTU (9.9 equivalents calculated from the loading of each crown) and activation e.g. HOBt (9.9 equivalents calculated from the loading of each crown), NMM (19.9 equivalents calculated from the loading of each crown) are accurately weighed into suitable containers.

The protected and activated Fmoc amino acid derivatives are then dissolved in DMF (500 μl for each macrocrown e.g. for 20 macrocrowns, 20 x 10 eq. x 7 μ moles of derivative would be dissolved in 10 ml DMF). The appropriate derivatives are then dispensed to the appropriate wells ready for commencement of the 'coupling cycle'. As a standard, coupling reactions are allowed to proceed for 6 hours. The coupled assembly was then washed as detailed below.

Coupling of L-lactic Acid

L-lactic acid (10eq per macrocrown) and HOBt (10eq per macrocrown) were dissolved in dichloromethane (450 μL per macrocrown) and cooled with ice-stirring to 0°C. Diisopropylcarbodiamide (10eq per macrocrown) in dichloromethane (50μL per macrocrown) was added and the mixture stirred at 0°C for 1hr.

The resultant symmetric anhydride solution was apportioned to the appropriate wells (500μL per well), the pin assembly for coupling, added and reaction left for 5hrs. The coupled assembly was then washed as detailed below.

Esterification Using Fmoc-Abu-F / 4-dimethylaminopyridine

Fmoc-Abu-F (10eq per macrocrown) was dissolved in dichloromethane (450μL per well) and the pin assembly for esterification soaked in the acid fluoride solution for 5mins. 4-dimethylaminopyridine (2eq per macrocrown) in dichloromethane (50μL per macrocrown) was added to each well and the reaction left at RT for 1hr followed by washing as detailed below.

Washing Following Coupling

If a 20% piperidine/DMF deprotection is to immediately follow the coupling cycle, then the multipin assembly is briefly shaken to remove excess solvent washed consecutively with (200 ml each), MeOH (5 minutes) and DMF (5 minutes) and de-protected. If the multipin assembly is to be stored or reacted further, then a full washing cycle consisting of brief shaking then consecutive washes with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) is performed.

Following these general methods, the single depsipeptides shown in **Table 1** were sequentially assembled by applying the appropriate coupling procedure at the correct cycle during synthesis.

Acidolytic Mediated Cleavage of Peptide-Pin Assembly

Acid mediated cleavage protocols are strictly performed in a fume hood. A polystyrene 96 well plate (1 ml/well) is labelled, then the tare weight measured to the nearest mg. Appropriate wells are then charged with a trifluoroacetic acid/triisopropylsilane (95:5, v/v, 600 μl) cleavage solution, in a pattern corresponding to that of the multipin assembly to be cleaved.

The multipin assembly is added, the entire construct covered in tin foil and left for 2 hours. The multipin assembly is then added to another polystyrene 96 well plate (1 ml/well) containing trifluoroacetic acid/triisopropylsilane (95:5, v/v, 600 μl) (as above) for 5 minutes.

Work up of Cleaved Peptides

The primary polystyrene cleavage plate (2 hour cleavage) and the secondary polystyrene plate (5 minute wash) are then placed in the SpeedVac and the solvents removed (minimum drying rate) for 90 minutes.

The contents of the secondary polystyrene plate are transferred to their corresponding wells on the primary plate using an acetonitrile/water/acetic acid (50:45:5, v/v/v) solution (3 x 150 μl) and the spent secondary plate discarded.

Analysis of Products

A 5μL aliquot from each well is diluted to 100 μl with 0.1% aq. TFA, then a 10 μL aliquot from this plate diluted with a further 100 μl 0.1% aq. TFA. The double diluted plate is analysed by HPLC-MS.

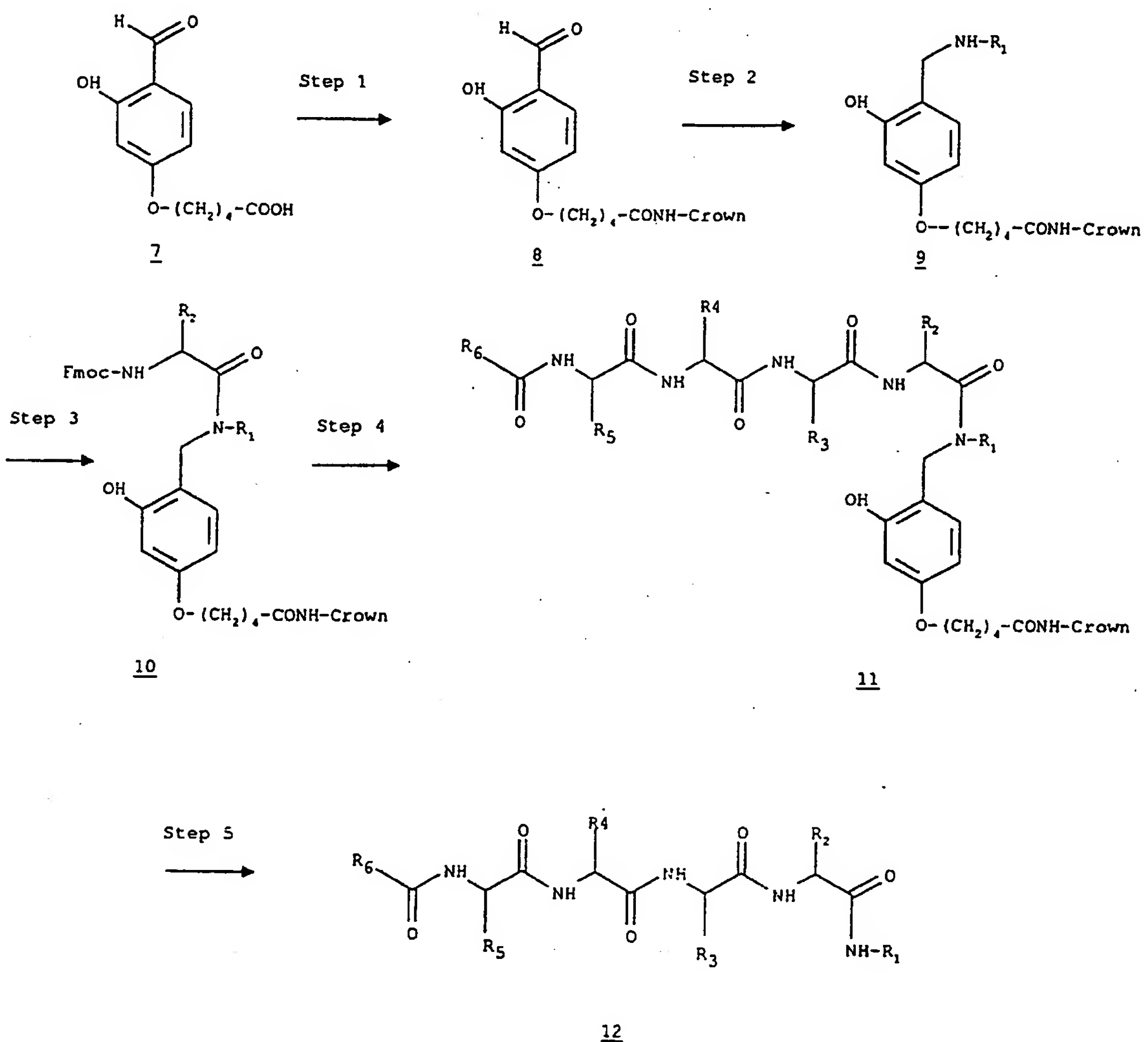
Final Lyophilisation of Peptides

The plate is covered with tin foil, held to the plate with an elastic band. A pin prick is placed in the foil directly above each well and the plate placed at -80°C for 30 minutes. The plate is then lyophilised on the 'Heto freeze drier' overnight.

Finally, the dried plate is weighed. The total cleaved peptide is quantified (by weight) and the average content of each peptide calculated. Since all the peptides present have originated from the same peptide-pin assembly, cleaved under identical conditions, it is reasonable to assume that the contents of each well are roughly equimolar.

Synthesis of Inhibitors of Hepatitis C NS3 protease

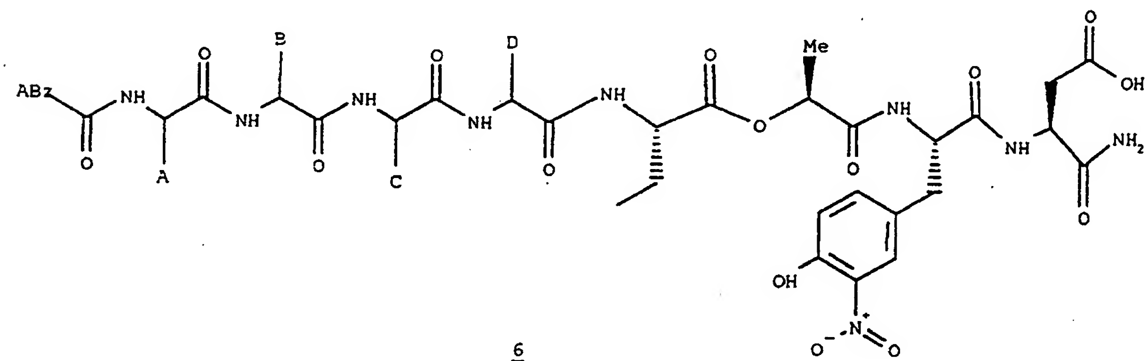
Inhibitors of the protease were synthesised according to the route shown in scheme 2.



Scheme 2

Depsipeptide Substrate Kinetics

The biological data for compounds of the type 6 (Scheme 1) are shown below in Table 1. Note that 2-aminobenzoyl-Glutamyl-Norleucyl-Glutamyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2, (Table 1) is a shorthand representation of N-aminobenzoyl-L-glutamyl -L-norleucinyl-L-glutamyl-L-2-aminobutanoyl-L-2-hydroxypropanoyl-L-3-nitrotyrosinyl-aspartyl amide 13 (Scheme 3) and is a specific example for the generalised structure 6 (Schemes 1 and 3). Other compounds described below are similarly represented.



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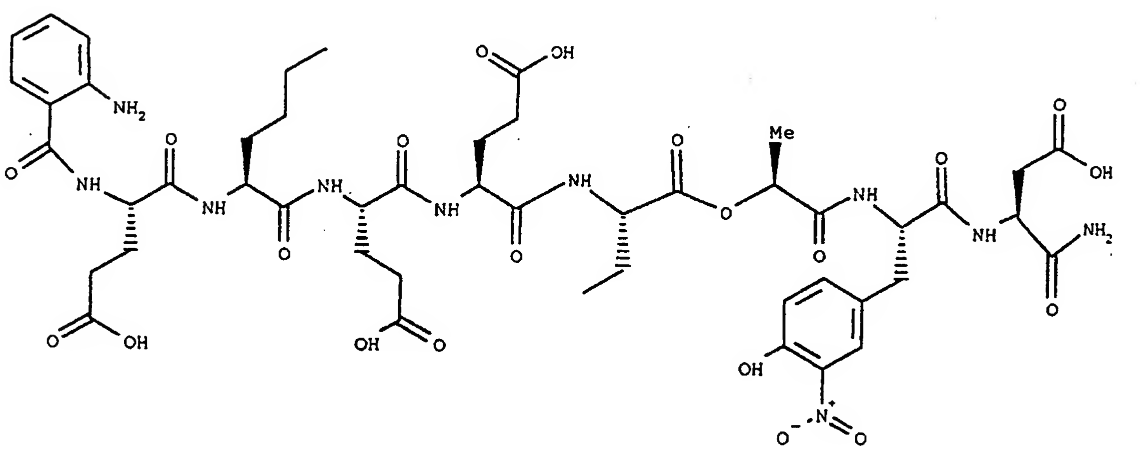

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Scheme 3

Table 1.

COMPOUND	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
2-aminobenzoyl-Glutamyl-Norleucyl-Glutamyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.2	7	480
2-aminobenzoyl-Glutamyl-Norleucyl-Glutamyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	12	830
2-aminobenzoyl-Glutamyl-Norleucyl-Valyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	4	2100
2-aminobenzoyl-Glutamyl-Norleucyl-Valyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.8	5	2700
2-aminobenzoyl-Glutamyl-Norleucyl-Serinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	10	830
2-aminobenzoyl-Glutamyl-Glutamyl-Glutamyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.2	14	240
2-aminobenzoyl-Glutamyl-Valyl-Serinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.7	16	730
2-aminobenzoyl-Glutamyl-Tyrosinyl-Serinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.3	9	560
2-aminobenzoyl-Glutamyl-Valyl-Norleucyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	10	1000
2-aminobenzoyl-<u>**D**</u>Glutamyl-Norleucyl-Valyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.7	7	1700
2-aminobenzoyl-<u>**D**</u>Glutamyl-Norleucyl-Glutamyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	37	230
2-aminobenzoyl-Glutaminyl-Valyl-Glutamyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.7	13	900
2-aminobenzoyl-Glutamyl-Norleucyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.7	9	1300
2-aminobenzoyl-Glutamyl-Norleucyl-3-Pyridylalanyl-Glutamyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	7	960
2-aminobenzoyl-Glutamyl-Valyl-3-Pyridylalanyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	6	1100
2-aminobenzoyl-Glutamyl-Glutamyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	5	1300
2-aminobenzoyl-Glutamyl-Valyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.9	10	1500
2-aminobenzoyl-Valyl-Norleucyl-3-Pyridylalanyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.3	13	380
2-aminobenzoyl-2-aminobutyryl-Norleucyl-3-Pyridylalanyl-Leucyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	130	80
2-aminobenzoyl-2-aminobutyryl-Glutamyl-3-Pyridylalanyl-Leucyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.3	8	630
2-aminobenzoyl-2-aminobutyryl-Valyl-3-Pyridylalanyl-Leucyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	14	480
2-aminobenzoyl-Valyl-Glutamyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-Nitrotyrosinyl-Aspartyl-NH_2	0.3	17	290
2-aminobenzoyl-Valyl-Valyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.2	30	110
2-aminobenzoyl-<u>**D**</u>- Glutamyl-Norleucyl-3-Pyridylalanyl-Leucyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	15	440
2-aminobenzoyl-<u>**D**</u>Glutamyl-Valyl-3-Pyridylalanyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.7	15	780

2-aminobenzoyl-Glutamyl-Norleucyl-3-Pyridylalanyl-Glutamyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.3	8	630
2-aminobenzoyl-Glutamyl-Norleucyl-Homophenylalanylnylalanyl-Leucyl- AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	5	1300
2-aminobenzoyl-Glutamyl-Norleucyl-Tyrosinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	9	1100
2-aminobenzoyl-DGlutamyl-Norleucyl-Tyrosinyl-Leucyl - AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	15	670
2-aminobenzoyl-DGlutamyl-Valyl-Tyrosinyl-Leucyl - AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	12	830
2-aminobenzoyl-Glutamyl-Valyl-Tyrosinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	1	10	1700
2-aminobenzoyl-Glutamyl-Norleucyl-Tyrosinyl-Glutamyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.4	4	1700
2-aminobenzoyl-Glutaminyl-Valyl-Tyrosinyl-Leucyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	1.9	70	450
2-aminobenzoyl-Glutamyl-Valyl-Tyrosinyl-Phenylalanyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	6	1400
2-aminobenzoyl-Glutamyl-Norleucyl-3-Pyridylalanyl-Prolyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	7	1200
2-aminobenzoyl-Glutamyl-Valyl-Tyrosinyl-Prolyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.6	8	1300
2-aminobenzoyl-Glutamyl-Norleucyl-Tyrosinyl-Prolyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.5	6	1400
2-aminobenzoyl- Glutamyl-Norleucyl-Valyl-Phenylalanyl-AbuΨ[COO]Ala-3-nitrotyrosinyl-Aspartyl-NH_2	0.8	5	2700

General Synthesis of Alkyl Amide Singles.

Preparation of Multipin Assembly

Whilst wearing standard plastic gloves, the Fmoc-Rink-DA/MDA macrocrowns are assembled (simply clipped) onto stems and slotted into the 8 x 12 stem holder in the desired pattern for synthesis.

Removal of Nα-Fmoc Protection

A 250 ml solvent resistant bath is charged with 200 ml of a 20% piperidine/DMF solution. The multipin assembly is added and deprotection allowed to proceed for 30 minutes. The assembly is then removed and excess solvent removed by brief shaking. The assembly is then washed consecutively with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) and left to air dry for 15 minutes.

Quantitative UV Measurement of Fmoc Chromophore Release

A 1 cm path length UV cell is charged with 1.2 ml of a 20% piperidine/DMF solution and used to zero the absorbance of the UV spectrometer at a wavelength of 290nm. A UV standard is then prepared consisting of 5.0 mg Fmoc-Asp(OBut)-Pepsyn KA (0.08 mmol/g) in 3.2 ml of a 20% piperidine/DMF solution. This standard gives Abs_{290} = 0.55-0.65 (at room temperature). An aliquot of the multipin deprotection solution is then diluted as appropriate to give a theoretical Abs_{290} = 0.6, and this value compared with the actual experimentally measured absorbance showing the efficiency of previous coupling reaction.

Coupling of 5(4-formyl-3-hydroxyphenoxy)pentanoic acid to pins

5(4-formyl-3-hydroxyphenoxy)pentanoic acid (10eq), 1-hydroxybenzotriazole.H_2O (10eq), BOP (9.95eq) and NMM (19.9eq) were dissolved in DMF (0.5mL per well) and agitated for 30secs. 500μL of solution was dispensed to each well of a 96-well polypropylene plate. 10 x H.-Gly-MA/DMA-Macrocrown loaded onto pins / pin holder was added to the acylation mixture containing wells and the reaction left for 4hrs.

The pin assembly was removed from the plate, shaken free of excess liquid then immersed in DMF (200mL) for 5mins. The assembly was again shaken then immersed in MeOH (200mL, 3 x 5mins) and allowed to air dry.

Coupling Of Amines to Backbone Aldehyde

The amines were dissolved in DMF /1% AcOH(450µL per well) and dispensed into appropriate wells. The pin assembly was then added and left for 5 minutes. After this time $Na(AcO)_3BH$ (10eq per well in 100µL DMF per well) was added and the reaction left 4hrs with occasional agitation to remove any gas bubbles formed.

The pin assembly was removed from the plate, shaken free of excess liquid then immersed in DMF / H_2O (200mL, 9:1, v/v) for 5mins. The acetate salt was neutralised by treatment of the pin assembly with 20% piperidine / DMF (200mL, v/v) for 30mins. The assembly was shaken then immersed DMF (200mL) for 5mins, then MeOH (200mL, 3 x 5mins) and allowed to air dry.

Coupling Of Symmetric Anhydride Amino Acid Residues To Aminated Resin

Amino acid (20eq) was dissolved in dichloromethane (3.5mL) / DMF (small quantity as required) and stirred / ice-cooled. Diisopropylcarbodiimide (10eq) was added and the mixture left to stir at $0^{o}C$ for 30mins, then warmed up to room temperature over 30 minutes. The solutions or gels were then dispensed as well as possible (Phe was a problem) into a 96 well plate as appropriate and the reactions left 20 hours in a saturated atmosphere of DCM. The coupled assembly was then washed as detailed below.

Coupling Of Standard Amino Acid Residues

Coupling reactions are performed by charging the appropriate wells of a polypropylene 96 well plate with the pattern of activated solutions required during a particular round of coupling. Macrocrown (approx 7 µmole) standard couplings are performed in DMF (500 µl).

Coupling of an Amino-acid Residue To Appropriate Well

Whilst the multipin assembly is drying, the appropriate N_α-Fmoc amino acid pfp esters (10 equivalents calculated from the loading of each crown) and HOBt (10 equivalents) required for the particular round of coupling are accurately weighed into suitable containers. Alternatively, the appropriate N_α-Fmoc amino acids (10 equivalents calculated from the loading of each crown), desired coupling agent e.g. HBTU (9.9 equivalents calculated from the loading of each crown) and activation e.g. HOBt (9.9 equivalents calculated from the loading of each crown), NMM (19.9 equivalents calculated from the loading of each crown) are accurately weighed into suitable containers.

The protected and activated Fmoc amino acid derivatives are then dissolved in DMF (500 μl for each macrocrown e.g. for 20 macrocrowns, 20 x 10 eq. x 7 µmoles of derivative would be dissolved in 10 000 µl DMF). The appropriate derivatives are then dispensed to the appropriate wells ready for commencement of the 'coupling cycle'. As a standard, coupling reactions are allowed to proceed for 6 hours. The coupled assembly was then washed as detailed below.

Washing Following Coupling

If a 20% piperidine/DMF deprotection is to immediately follow the coupling cycle, then the multipin assembly is briefly shaken to remove excess solvent washed consecutively with (200 ml each), MeOH (5 minutes) and DMF (5 minutes) and de-protected (see 6.2). If the multipin assembly is to be stored or reacted further, then a full washing cycle consisting brief shaking then consecutive washes with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) is performed.

Coupling Of Benzoic Anhydride To Last Residue

Benzoic Anhydride (20eq) is dissolved in DMF (500 μl for each macrocrown e.g. for 20 macrocrowns, 20 x 10 eq. x 7 µmoles of derivative would be dissolved in 10 000 µl DMF) to which NMM (40eq) was added. The solution is then dispensed to the appropriate wells ready for commencement of the 'coupling cycle'.The reaction was then left for 2 hours. The coupled assembly was then washed as detailed below and treated with 20% piperidine in DMF followed by the standard washing cycle before cleavage.

Following these general methods, the single peptide inhibitors shown in **Table 2** were sequentially assembled by applying the appropriate coupling procedure at the correct cycle during synthesis.

Acidolytic Mediated Cleavage of Peptide-Pin Assembly

Acid mediated cleavage protocols are strictly performed in a fume hood. A polystyrene 96 well plate (1 ml/well) is labelled, then the tare weight measured to the nearest mg. Appropriate wells are then charged with a trifluoroacetic acid/triisopropylsilane (95:5, v/v, 600 µl) cleavage solution, in a pattern corresponding to that of the multipin assembly to be cleaved.

The multipin assembly is added, the entire construct covered in tin foil and left for 2 hours. The multipin assembly in then added to another polystyrene 96 well plate (1 ml/well) containing trifluoroacetic acid/triisopropylsilane (95:5, v/v, 600 µl) (as above) for 5 minutes.

Work up of Cleaved Peptides

The primary polystyrene cleavage plate (2 hour cleavage) and the secondary polystyrene plate (5 minute wash) are then placed in the GeneVac and the solvents, no heating required.

The contents of the secondary polystyrene plate are transferred to their corresponding wells on the primary plate using an acetonitrile/water/acetic acid (50:45:5, v/v/v) solution (3 x 150 µl) and the spent secondary plate discarded.

Analysis of Products

A 5µL aliquot from each well is diluted to 100 µl with 0.1% aq. TFA, then a 10µL aliquot from this plate diluted with a further 100 µl 0.1% aq. TFA. The double diluted plate is analysed by HPLC-MS.

Final Lyophilisation of Peptides

The plate is covered with tin foil, held to the plate with an elastic band. A pin prick is placed in the foil directly above each well and the plate placed at -80°C for 30 minutes. The plate is then lyophilised on the 'Heto freeze drier' overnight.

Finally, the dried plate is weighed. The total cleaved peptide is quantified (by weight) and the average content of each peptide calculated. Since all the peptides present have originated from the same peptide-pin assembly, cleaved under identical conditions, it is reasonable to assume that the contents of each well are roughly equimolar.

Inhibitor names and lab numbers:

24-14) N-benzoyl-L-glutamyl -L-norleucyl -L-homophenylalanyl -L-leucyl - 3-methyl butan -1- amide [SEQ ID 1]

24-22) N-benzoyl-L-glutamyl -L-norleucyl -L-homophenylalanyl -L-phenyl - 3-methyl butan -1- amide [SEQ ID 2]

24-38) N-benzoyl-L-glutamyl -L-norleucyl -L-homophenylalanyl -L-leucyl - 2-phenyl ethyl -1- amide [SEQ ID 3]

24-46) N-benzoyl-L-glutamyl -L-norleucyl -L-homophenylalanyl -L-phenyl - 2-phenyl ethyl -1- amide [SEQ ID 4]

24-15) N-benzoyl-L-glutamyl -L-norleucyl -1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -L-leucyl - 3-methyl butan -1- amide [SEQ ID 5]

24-19) N-benzoyl-L-glutamyl -L-norleucyl -1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -L-glutamyl - 3-methyl butan -1- amide [SEQ ID 6]

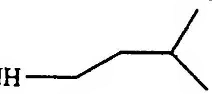
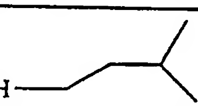
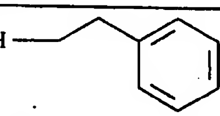
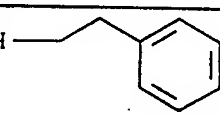
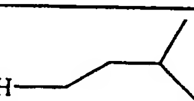
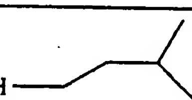
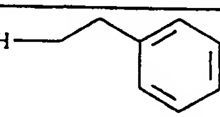
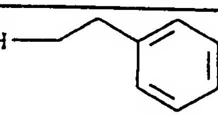
24-23) N-benzoyl-L-glutamyl -L-norleucyl -1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -phenyl- 3-methyl butan -1- amide [SEQ ID 7]

24-39) N-benzoyl-L-glutamyl -L-norleucyl -1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -L-leucyl - 2-phenyl ethyl -1- amide [SEQ ID 8]

24-47) N-benzoyl-L-glutamyl -L-norleucyl 1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -L-phenyl - 2-phenyl ethyl -1- amide [SEQ ID 9]

24-95) N-benzoyl-L-glutamyl -L-norleucyl -1,2,3,4-D-tetrahydroisoquinoline-3-carboxamidyl -L-phenyl - ethyl -1- amide [SEQ ID 10]

Table 3.

No.	Compound	EC_{50}	K_I(app)	K_I(um)
24-14	Bz-Glu-Nle-hPhe-Leu-NH 	102.4	16.3	5.4
24-22	Bz-Glu-Nle-hPhe-Phe-NH 	104.3	52.6	17.5
24-38	Bz-Glu-Nle-hPhe-Leu-NH 	155.3	38.9	13.0
24-46	Bz-Glu-Nle-hPhe-Phe-NH 	111.0	29.0	9.7
24-15	Bz-Glu-Nle-DTic-Leu-NH 	162.1	100.0	33.3
24-19	Bz-Glu-Nle-DTic-Glu-NH 	403.2	270.0	90.1
24-23	Bz-Glu-Nle-DTic-Phe-NH 	131.4	65.3	21.8
24-39	Bz-Glu-Nle-DTic-Leu-NH 	346.9	150.0	50.0
24-47	Bz-Glu-Nle-DTic-Phe-NH 	172.9	69.5	23.2
24-95	Bz-Glu-Nle-DTic-Phe-NH 	798.8	100.0	33.3

Pharmacophoric Profile Definition and Specification

Methodology

Collections of compounds with biological activity for HCV NS3 J8 are provided as training sets. Each compound in a training set undergoes full conformational analysis[12]. A representative number of conformers are generated over a given energy range above the lowest energy conformation[13,14].

This information is used to derive a pharmacophore (based on seven chemical feature type rules)[15] that correlates to the observed biological activity. It is assumed that the molecules in a training set all act at the same target in the same manner of action.

From the available data, three motifs are identified that correspond to compounds that are recognised by HCV NS3 J8 as substrates. These motifs are as follows:

MOTIF 1 Negative Ionizable - Hydrophobe - Hydrophobe - Hydrophobe

MOTIF 2 Negative Ionizable - Hydrophobe - Aromatic - Hydrophobe

MOTIF 3 Negative Ionizable - Hydrophobe - Aromatic or Hydrophobe - Negative Ionizable

These represent the chemical functionality required at positions P5 - P4 - P3 - P2 in the HCV NS3 J8 active site.

A HYDROPHOBE feature is defined as

* a contiguous set of atoms that are not adjacent to any concentrations of charge (charged atoms or electronegative atoms), in a conformation such that the atoms have surface accessibility, including phenyl,

cycloalkyl, isopropyl and methyl.

* this may also include residues which have a partial hydrophobic character such as Lysyl or Glutaminyl amino acid sidechains.

A NEGATIVE IONIZABLE feature is defined as

* atoms or groups of atoms that are likely to be deprotonated at physiological pH, such as trifluromethyl sulfonamide hydrogens, sulfonic acids (centroid of the three oxygens), phosphonic acids (centroid of the three oxygens), sulphinic, carboxylic or phosphinic acids (centroid of the two oxygens), tetrazoles and negative charges not adjacent to a positive charge).

A RING AROMATIC feature is defined as

* matching aromatic rings with five or six member atoms

A pharmacophore* consisting of at least the following chemical features can be used to describe MOTIF 1:

Three HYDROPHOBE[11] features and a NEGATIVE IONIZABLE feature. The HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms). The NEGATIVE IONIZABLE feature is similarly represented by a sphere 1.7 Angstroms radius (up to 2.7 Angstroms).

see figure 1.

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of -8.207, -3.059, -3.78
* Hydrophobe 2 has co-ordinates of -2.975, 4.725, -0.229

* Hydrophobe 3 has co-ordinates of 6.065, 2.205, 3.991
* Hydrophobe 4 has co-ordinates of 3.385, -2.935, -1.149

The distance and angle constraints for these are described in figure 2:

see figure 2.

The tolerances on all distances between these features is +/- 0.5 Angstroms and the geometric angles +/- 20 Degrees.

* In this context, the use of the term "pharmacophore" is not meant to imply any pharmacological activity. The term refers to those chemical features and their distribution in three-dimensional space which constitute and epitomise the preferred requirements for molecular interaction with the receptor. In this case the receptor being the catalytic active site of the protease HCV NS3 J8.

Similarly, a pharmacophore consisting of at least the following chemical features can be used to describe MOTIF 2:

Two HYDROPHOBE features, a NEGATIVE IONIZABLE feature and a RING AROMATIC feature.
The HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms). The NEGATIVE IONIZABLE feature is similarly represented by a sphere 1.7 Angstroms radius (up to 2.7 Angstroms). The RING AROMATIC is represented as two equal size spheres (1.6 Angstroms radius up to 2.0 Angstoms) whose centroids are 3.1 Angstroms apart. One sphere corresponds to the position of an aromatic ring moiety and the other to the projected point of the electron pi stacking of the aromatic ring system.

see figure 3.

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of 4.907, -1.284, 3.039
* Hydrophobe 2 has co-ordinates of 1.496, 3.212, -3.793
* Hydrophobe 3 has co-ordinates of -4.324, -4.228, -3.313
* Ring Aromatic centroid 4 has co-ordinates of -0.798, -1.230, 2.330
* Ring Aromatic projected point 5 has co-ordinates of -4.324, -4.228, -3.313

The distance and angle constraints for these are described in figures 4 and 5:

see figures 4 and 5.

The tolerances on all distances between these features is +/- 0.5 Angstroms and the geometric angles +/- 20 Degrees.

A pharmacophore consisting of at least the following chemical features can be used to describe MOTIF 3:

Two HYDROPHOBE features and two NEGATIVE IONIZABLE features. The HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms). The NEGATIVE IONIZABLE features are similarly represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms).

see figure 6.

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of -8.551, 0.769,

-0.895

* Hydrophobe 2 has co-ordinates of -0.697, 1.087, -5.655
* Negative Ionizable 3 has co-ordinates of 6.098, 1.653, 4.709
* Hydrophobe 4 has co-ordinates of 0.503, -2.453, 2.784

The distance and angle constraints for these are described in figure 7:

see figure 7.

Compounds that exemplify the pharmacophore

Bz-Glu-Nle-hphe-Leu-R where R = 3-methyl butylamine shown mapped to MOTIF 1 and Bz-Glu-Nle-hphe-Phe-R where R = 2-phenylethyl amine is shown mapped to MOTIF 2 and Bz-Glu-Nle-dTic-Glu-R where R = 3-methyl butylamine shown mapped to MOTIF 3.

see figures 8, 9 and 10.

Those skilled in the art will appreciate that the compounds of the invention may essentailly consist of an amino acid (aa) sequence (or non peptide mimetic thereof) or may include a sequence corresponding to one of the pharmacophoric motifs described herein. For example the sequence might consist of or include the sequence $[aa]_n$ wherein n is any integer from 4 upwards, for example wherein n is 4,5,6,7,8,9,10,11 or 12.

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Claims

1. A compound having biological activity as an inhibitor of Hepatitis C NS3 protease, which compound is recognised by HCV NS3 J8 as a substrate, and having at least four chemical functionalities for interacting with at least the P_5-P_4-P_3-P_2 pockets of the HCV NS3 J8 catalytically active site, which functionalities are each provided by an amino acid residue (or a wholly or partly non-peptide mimetic thereof), wherein said functionalities provide a pharmacophoric motif selected from the group consisting of:

MOTIF 1 Negative Ionizable - Hydrophobe - Hydrophobe - Hydrophobe

MOTIF 2 Negative Ionizable - Hydrophobe - Aromatic - Hydrophobe

MOTIF 3 Negative Ionizable - Hydrophobe - Aromatic or Hydrophobe - Negative Ionizable

wherein;

a HYDROPHOBE feature is defined as

* a contiguous set of atoms that are not adjacent to any concentrations of charge (charged atoms or electronegative atoms), in a conformation such that the atoms have surface accessibility, including phenyl, cycloalkyl, isopropyl and methyl, (this may also include residues which have a partial hydrophobic character such as Lysyl or Glutaminyl amino acid sidechains)

and;

a NEGATIVE IONIZABLE feature is defined as

* atoms or groups of atoms that are likely to be deprotonated at physiological pH, such as trifluromethyl sulfonamide hydrogens, sulfonic acids (centroid of the three oxygens), phosphonic acids (centroid of the three oxygens), sulphinic, carboxylic or phosphinic acids (centroid of the two oxygens), tetrazoles and negative charges not adjacent to a positive charge)

and;

a RING AROMATIC feature is defined as

* matching aromatic rings with five or six member atoms

and wherein;

(i) a pharmacophore consisting of at least the following chemical features can be used to describe MOTIF 1:

Three HYDROPHOBE features and a NEGATIVE IONIZABLE feature, in which the HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms); the NEGATIVE IONIZABLE feature is similarly represented by a sphere 1.7 Angstroms radius (up to 2.7 Angstroms);

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of -8.207, -3.059, -3.78
* Hydrophobe 2 has co-ordinates of -2.975, 4.725, -0.229
* Hydrophobe 3 has co-ordinates of 6.065, 2.205, 3.991
* Hydrophobe 4 has co-ordinates of 3.385, -2.935, -1.149

and the distance and angle constraints for these are described in figure 2:

and wherein;

(ii) a pharmacophore consisting of at least the following chemical features can be used to describe MOTIF 2:

Two HYDROPHOBE features, a NEGATIVE IONIZABLE feature and a RING AROMATIC feature, in which the HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms); the NEGATIVE IONIZABLE feature is similarly represented by a sphere 1.7 Angstroms radius (up to 2.7 Angstroms); the RING AROMATIC is represented as two equal size spheres (1.6 Angstroms radius up to 2.0 Angstroms) whose centroids are 3.1 Angstroms apart, one

sphere corresponds to the position of an aromatic ring moiety and the other to the projected point of the electron pi stacking of the aromatic ring system;

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of 4.907, -1.284, 3.039
* Hydrophobe 2 has co-ordinates of 1.496, 3.212, -3.793
* Hydrophobe 3 has co-ordinates of -4.324, -4.228, -3.313
* Ring Aromatic centroid 4 has co-ordinates of -0.798, -1.230, 2.330
* Ring Aromatic projected point 5 has co-ordinates of -4.324, -4.228, -3.313

and distance and angle constraints for these are described in figures 4 and 5:

and wherein;
(iii) a pharmacophore consisting of at least the following chemical features can be used to describe MOTIF 3:

Two HYDROPHOBE features and two NEGATIVE IONIZABLE features, in which the HYDROPHOBE features are represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms); the NEGATIVE IONIZABLE features are similarly represented by spheres 1.7 Angstroms radius (up to 2.7 Angstroms);

The absolute sphere centroid positions of each feature are described as follows:

* Negative Ionizable 1 has Cartesian XYZ co-ordinates of -8.551, 0.769, -0.895
* Hydrophobe 2 has co-ordinates of -0.697, 1.087, -5.655
* Negative Ionizable 3 has co-ordinates of 6.098, 1.653, 4.709
* Hydrophobe 4 has co-ordinates of 0.503, -2.453, 2.784

and the distance and angle constraints for these are described in figure 7:

and wherein;
(iv) the tolerances on all distances between features are +/- 0.5 Angstroms and the tolerances on all geometric angles are +/- 20 degrees for all three Motifs.

2. A compound according to claim 1 selected from the group consisting of SEQ ID Nos. 1-10, or a wholly or partly non peptide mimetic thereof, or N- or C-terminal derivatives thereof, or analogues thereof by virtue of conservative amino acid deletion, addition or substitution.

3. A composition which comprises a pharmaceutically acceptable amount of an inhibitor according to claim 1 or 2 together with a pharmaceutically acceptable carrier or diluent.

4. Use of an inhibitor according to claim 1 or 2 or a composition according to claim 3 in the manufacture of a medicament for the treatment of hepatitis C.

5. Use of an inhibitor according to claim 1 or 2 or a composition according to claim 3 for the treatment of hepatitis C.

6. A method of treatment of hepatitis C which comprises administering to a patient a pharmaceutically active amount of an inhibitor according to claim 1 or 2 or a composition according to claim 3.

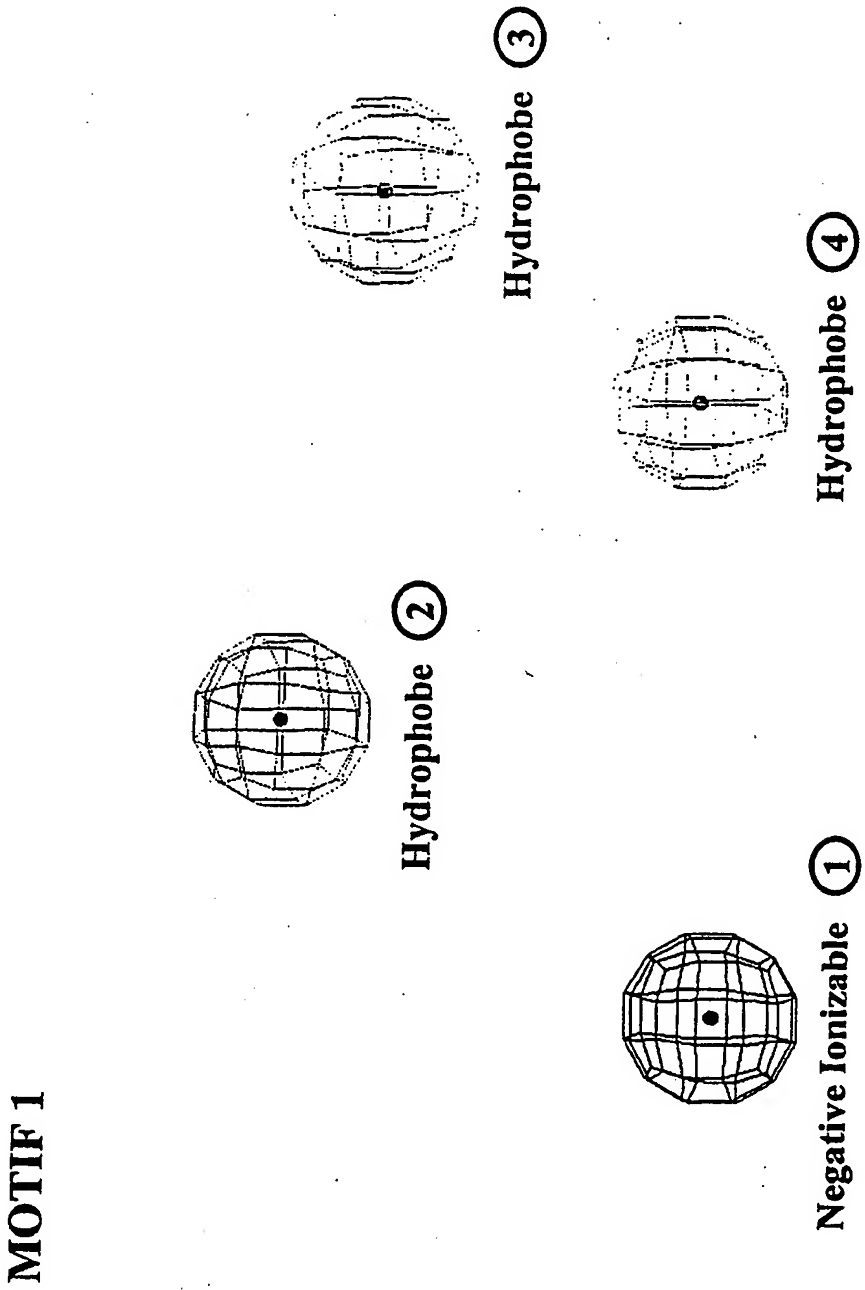

MOTIF 1
Hydrophobe 3
Hydrophobe 4
Hydrophobe 2
Negative Ionizable 1

Figure 1.

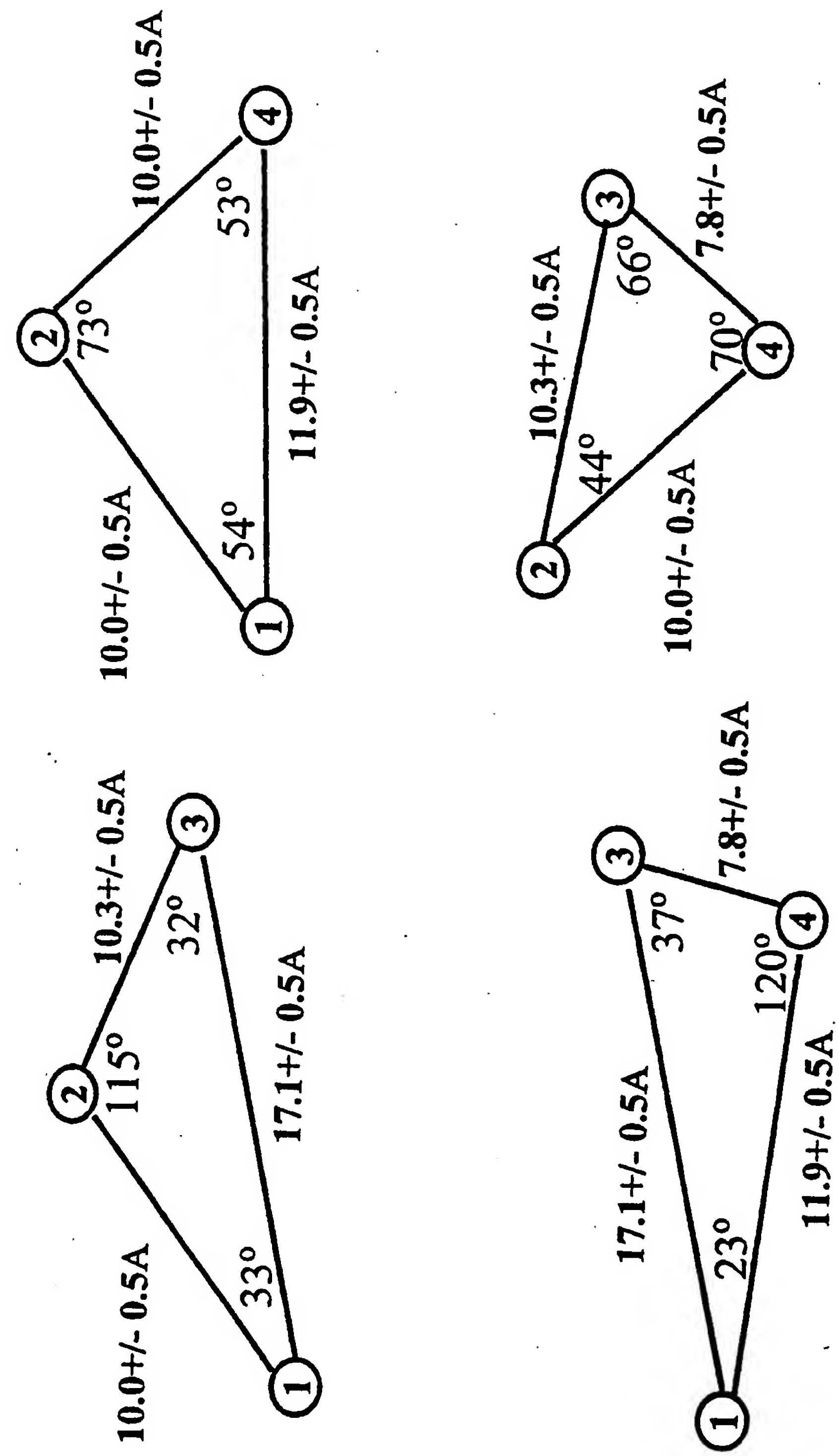

10.0+/- 0.5A
53°
73°
11.9+/- 0.5A
54°
10.0+/- 0.5A
10.3+/- 0.5A
66°
7.8+/- 0.5A
70°
44°
10.0+/- 0.5A
10.3+/- 0.5A
32°
115°
17.1+/- 0.5A
33°
10.0+/- 0.5A
7.8+/- 0.5A
37°
120°
17.1+/- 0.5A
23°
11.9+/- 0.5A

Figure 2.

MOTIF 2

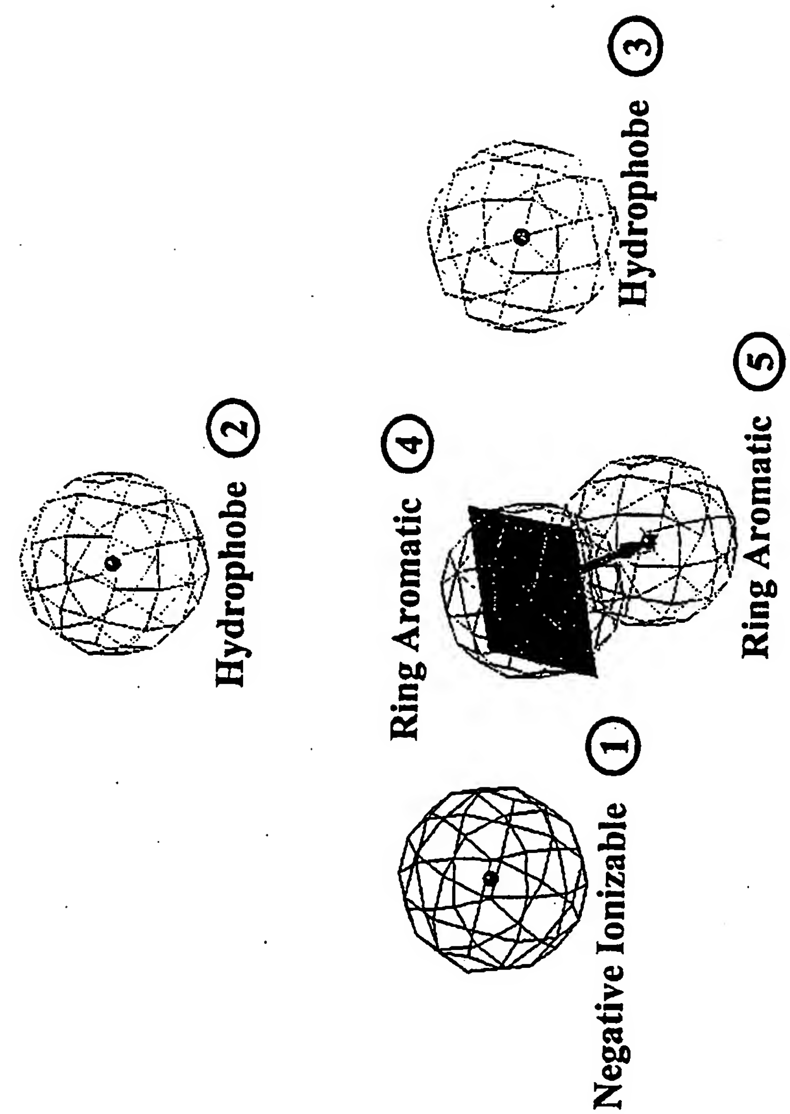


Figure 3.

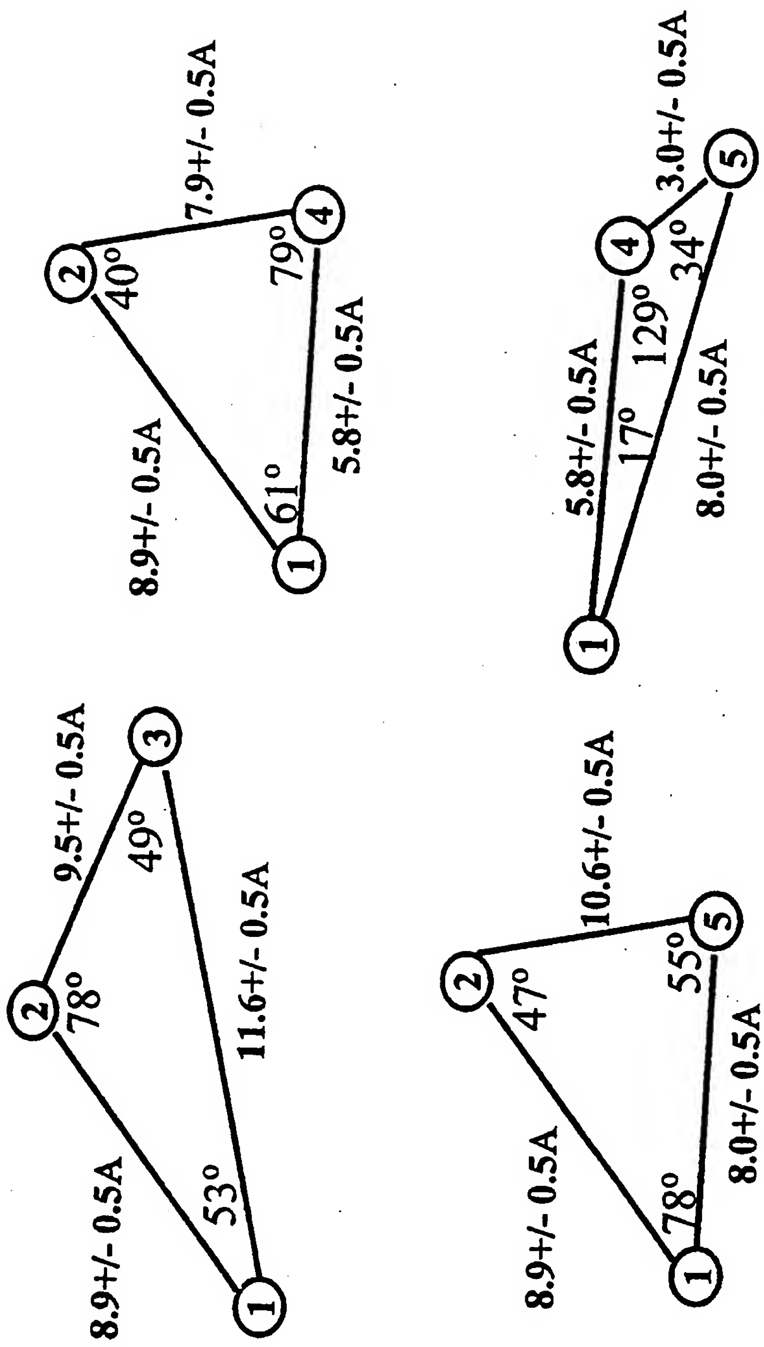

2
4
1
40°
79°
61°
7.9+/- 0.5A
5.8+/- 0.5A
8.9+/- 0.5A
4
5
1
129°
34°
17°
3.0+/- 0.5A
5.8+/- 0.5A
8.0+/- 0.5A
2
3
1
78°
49°
53°
9.5+/- 0.5A
11.6+/- 0.5A
8.9+/- 0.5A
2
5
1
47°
55°
78°
10.6+/- 0.5A
8.0+/- 0.5A
8.9+/- 0.5A

Figure 4.

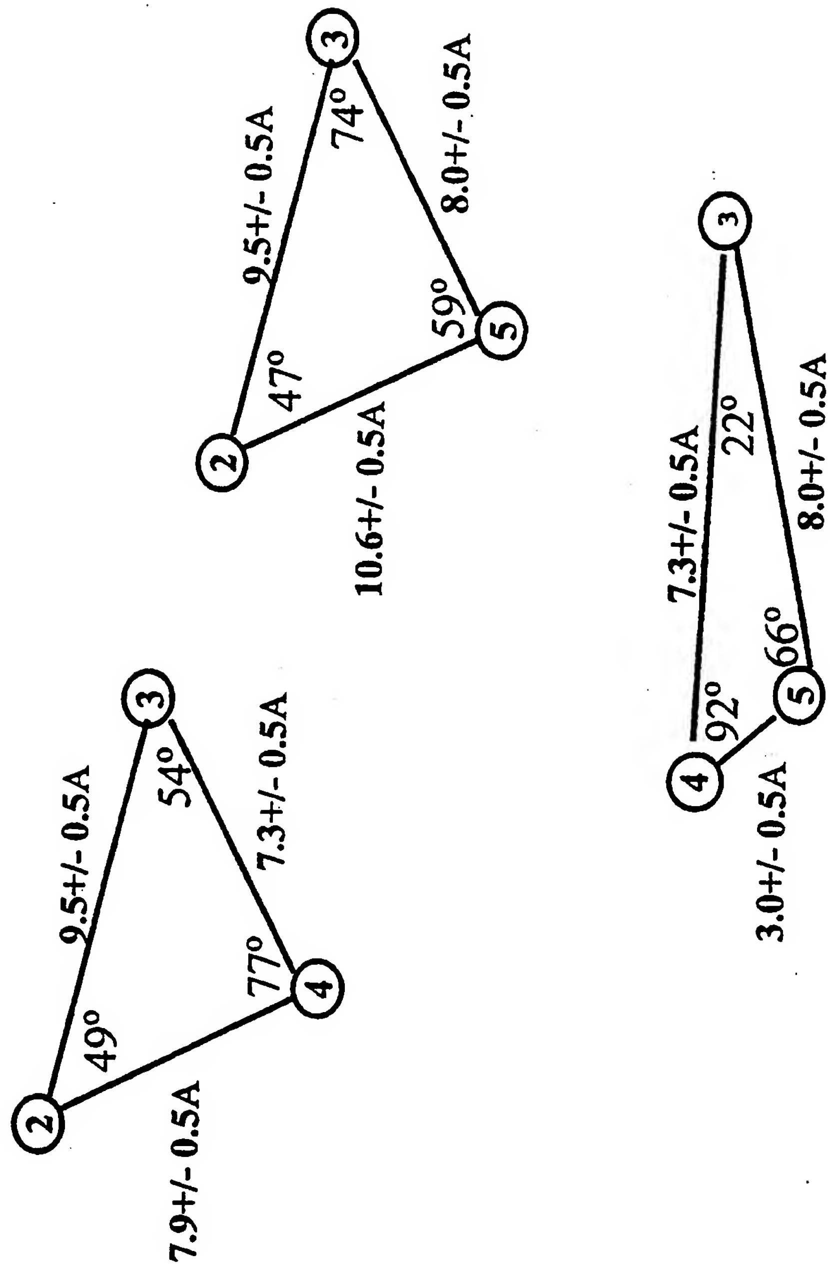

3
2
5
9.5+/- 0.5A
8.0+/- 0.5A
10.6+/- 0.5A
74°
59°
47°
3
5
4
7.3+/- 0.5A
8.0+/- 0.5A
3.0+/- 0.5A
22°
66°
92°
3
2
4
9.5+/- 0.5A
7.3+/- 0.5A
7.9+/- 0.5A
54°
77°
49°

Figure 5.

MOTIF 3

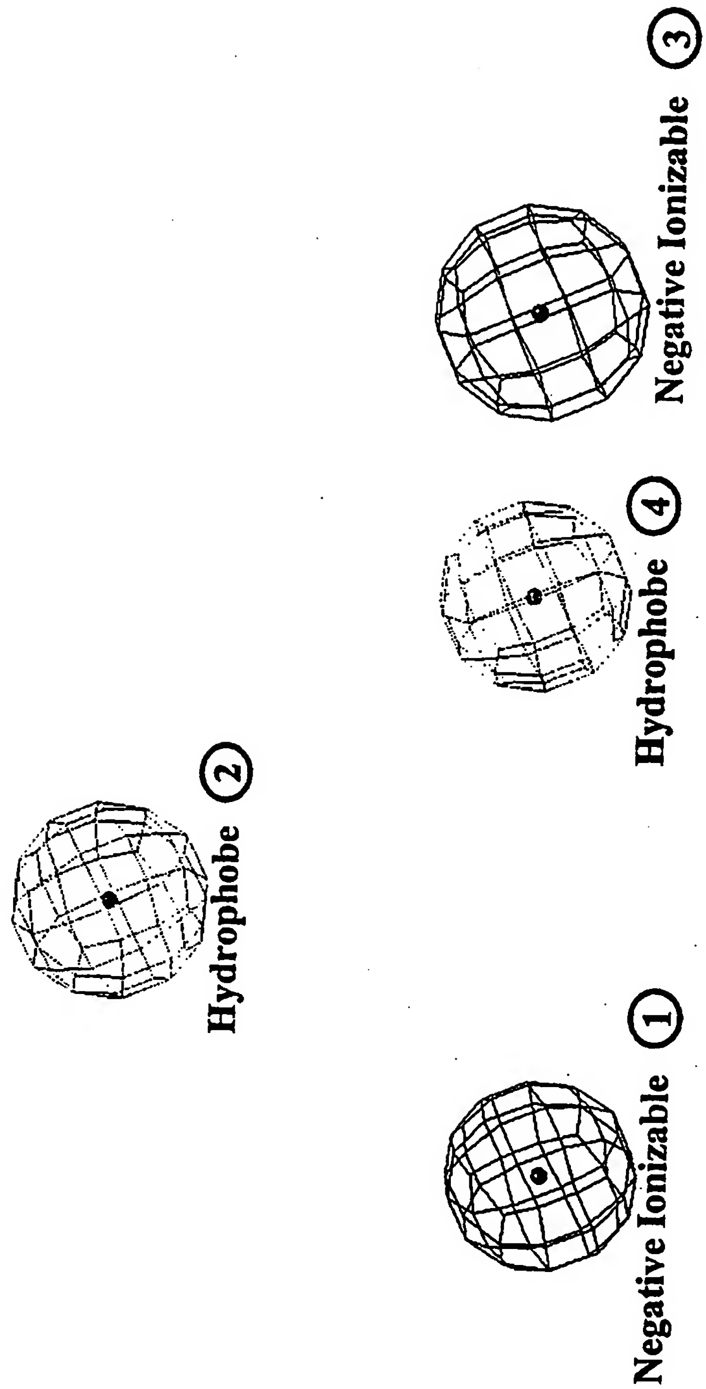


Figure 6.

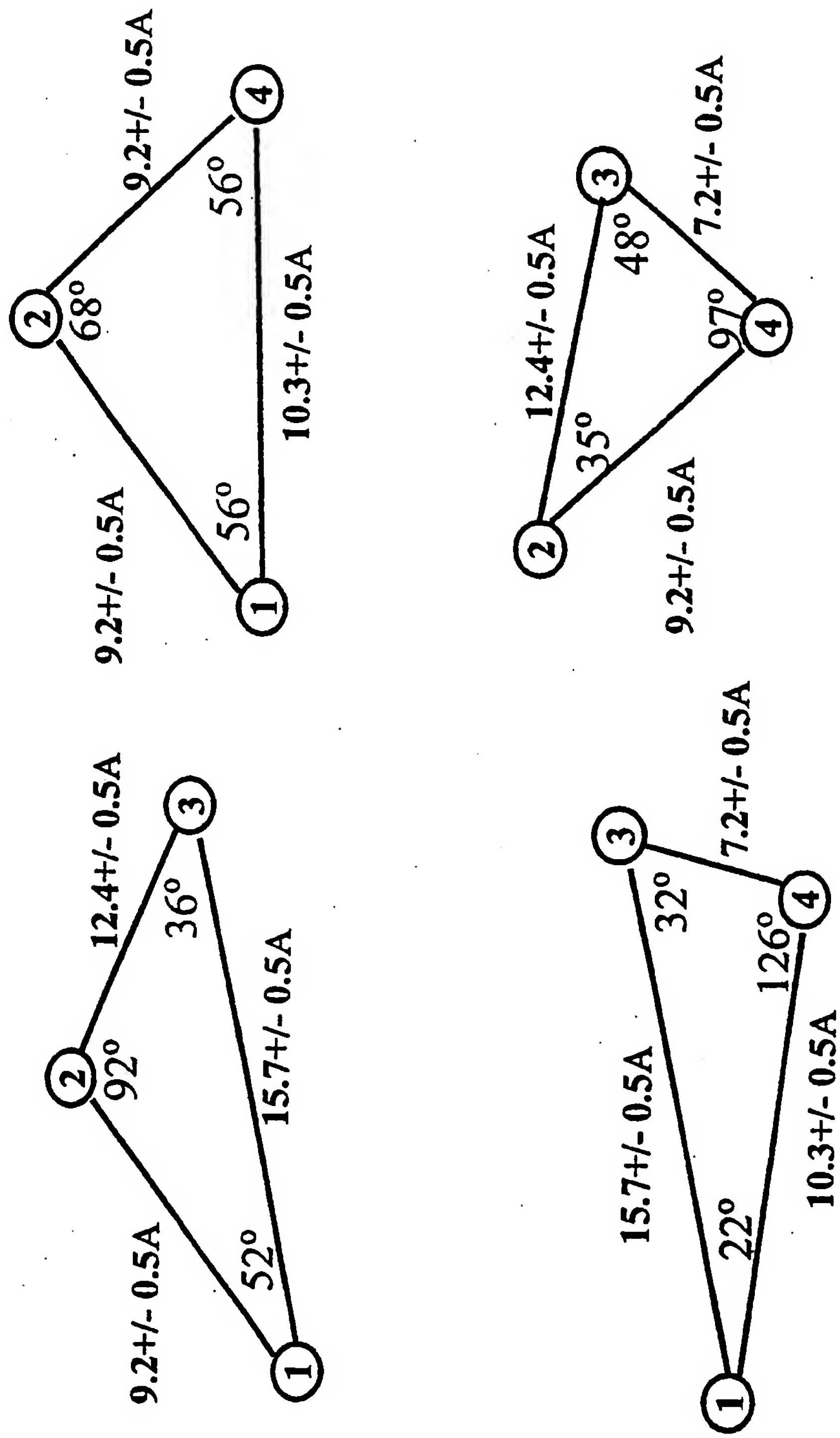

1
2
3
4
9.2+/- 0.5A
10.3+/- 0.5A
12.4+/- 0.5A
15.7+/- 0.5A
7.2+/- 0.5A
56°
68°
48°
97°
35°
36°
92°
52°
32°
126°
22°

Figure 7.

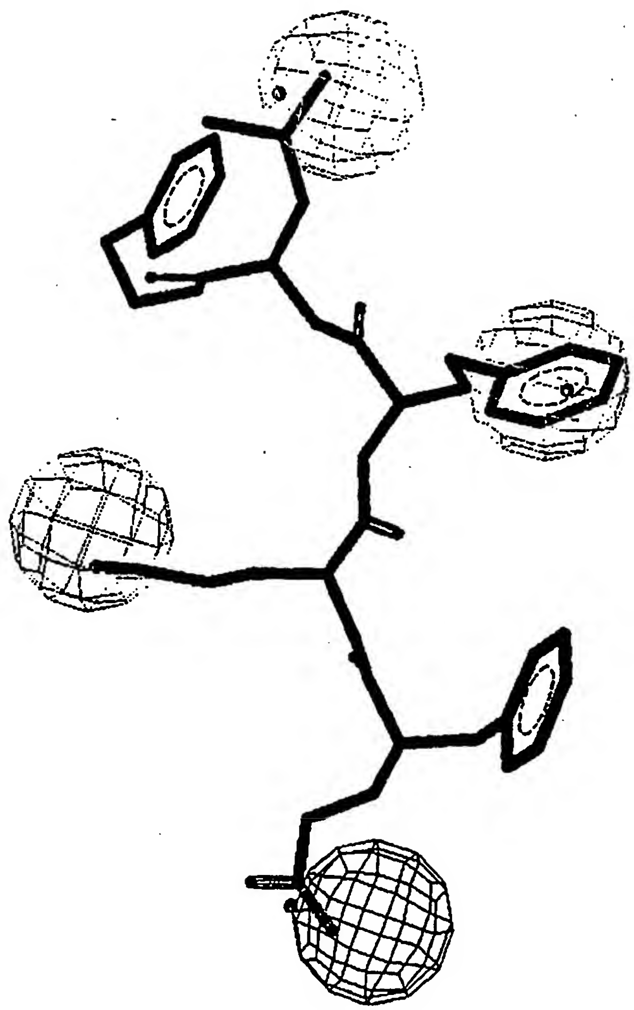
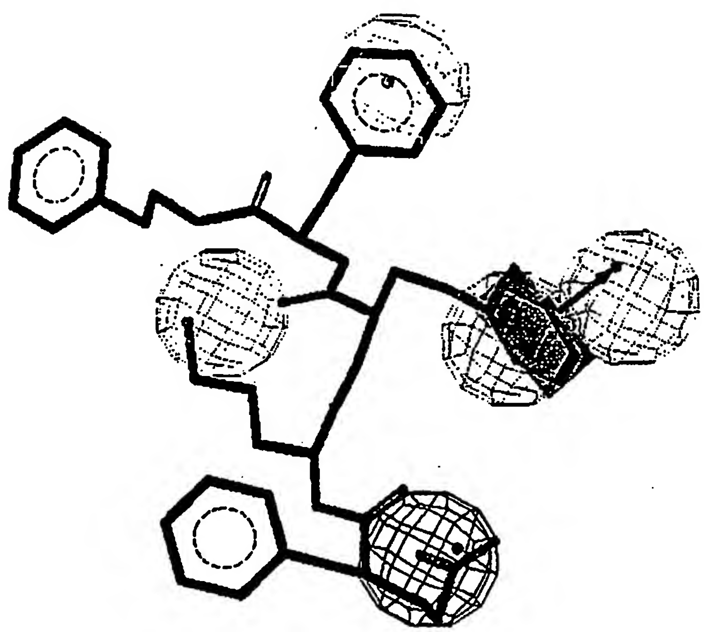


Figure 8.

Figure 9.



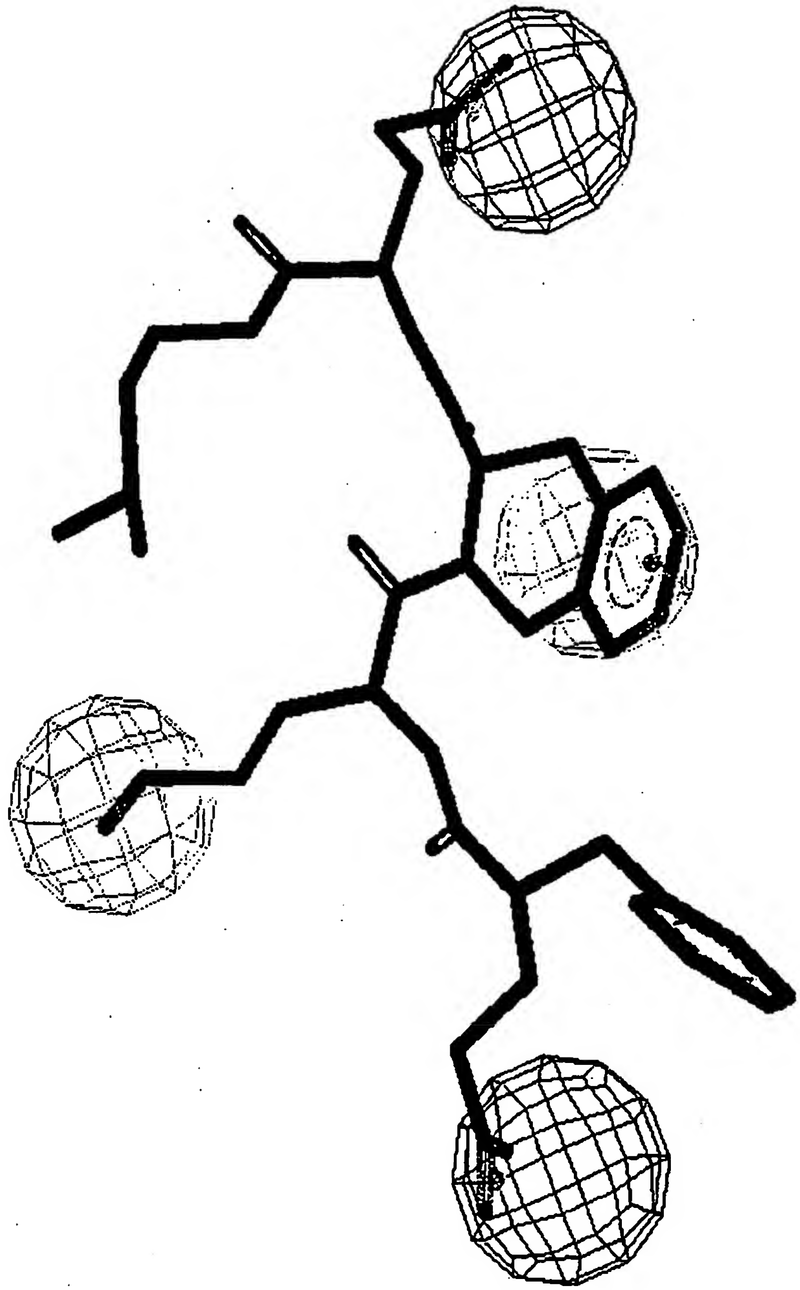


Figure 10.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01126

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/113 A61K38/07

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 13904 A (CEPHALON INC) 19 September 1991 see figure 16H ---	1-3
A	STEINKUHLER C ET AL: "IN VITRO ACTIVITY OF HEPATITIS C VIRUS PROTEASE NS3 PURIFIED FROM RECOMBINANT BACULOVIRUS-INFECTED SF9 CELLS" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 11, 15 March 1996, pages 6367-6373, XP002018451 cited in the application see figure 4 ---	1-6

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 1998	18/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MIN CHU ET AL: "STRUCTURE OF SCH 68631: A NEW HAPATITIS C VIRUS PROTEINASE INHIBITOR FROM STREPTOMYCES SP" TETRAHEDRON LETTERS, vol. 37, no. 40, 30 September 1996, pages 7229-7232, XP000627717 cited in the application see the whole document	1-6
A	WO 97 08304 A (ANGELETTI P IST RICHERCHE BIO ;STEINKUEHLER CHRISTIAN (IT); PESSI) 6 March 1997 cited in the application see the whole document	1-6
E	WO 98 17679 A (DEININGER DAVID D ;MURCKO MARK A (US); VERTEX PHARMA (US); FARMER) 30 April 1998 see the whole document	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/01126

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Although claims 5 and 6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   1-6 (ALL PARTIALLY)

   SEE FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01126

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9113904 A	19-09-1991	AU 661270 B	20-07-1995
		AU 7465491 A	10-10-1991
		CA 2077665 A	06-09-1991
		EP 0518955 A	23-12-1992
		EP 0732399 A	18-09-1996
		FI 923983 A	04-09-1992
WO 9708304 A	06-03-1997	IT RM950573 A	24-02-1997
		AU 6668696 A	19-03-1997
		EP 0846164 A	10-06-1998
WO 9817679 A	30-04-1998	AU 5147798 A	15-05-1998